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(57) Abstract

The present invention provides synthetic mucoadhesive liposomes and methods of using and preparing such liposomes. Also provided are mucoadhesive liposomes which comprise a barrier lipid derived from a mucosal surface.

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GLYCOSYLCERAMIDE-CONTAINING LIPOSOMES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is based on provisional patent application no. 60/042,449, filed in the United States Patent and Trademark Office on March 31, 1997, and hereby claims all benefits that are legally served thereby. The entire contents of the provisional patent application no. 60/042,449 are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates generally to the field of liposomes. More particularly, it provides synthetic glycosylceramide-containing liposomes for use in oral drug delivery. Also provided are liposomes which comprise a specific barrier lipid composition which is analogous to that of a target mucosal surface. A range of glycosylceramide concentrations are provided that, when combined with other lipids, form synthetic liposomal vesicles that adhere to mucosa. Liposomes having a glycosylceramide content outside the effective range either do not form intact vesicles, or do not adhere to the muscosal surface. The liposomes of the present invention are advantageous in that their preparation is simple, cost effective and therapeutically useful. Methods of local and systemic drug delivery and treatment using, the liposomes are also included within the invention.

BACKGROUND OF THE INVENTION

There is growing concern over systemic delivery of pharmaceutical agents by parenteral routes because of the invasive nature of the process and, particularly, the risk of infection. However, oral delivery via the gut is also unsuitable for many molecules, particularly those sensitive to acid or proteases. On the other hand, the skin although accessible and offering a large area for absorption, represents a relatively

impermeable and poorly vascularized tissue for systemic drug delivery (P.W. Wertz, *et al., Adv. Drug Delivery Rev.* **12**, 1 (1993)). In contrast, mucosal surfaces are richly vascularized and present an attractive locus for drug delivery.

For more than 100 years nitroglycerin has been administered sublingually, where it is absorbed through the floor of the mouth and ventral tongue so as to achieve systemic delivery (C.A. Squier, *et al., Crit. Rev. Oral Biol. Med.* **2**, 13 (1991)). A number of other drugs, generally in the form of quick-dissolving tablets, are now administered through sublingual or buccal routes; these include isosorbide dinitrate, nifedipine, captopril, buprenorphine, morphine, nalbuphine, alphaprodine, pethidine and nicotine (Robinson *et al. The Lancet* 666, (1987); Harris *et al., J. Pharmaceut. Sciences* **81**, 1 (1992); Hoogstraate *et al., Adv. Drug. Delivery Rev.* **12**, 99 (1993)). In all of these examples, administration of the drug, is followed by a rapid increase in plasma concentration.

In addition to the oral mucosa, many of an organism's other mucosal surfaces are accessible to varying degrees, and as these tissues are richly vascularized, there has been increasing interest in transmucosal drug delivery vehicles and mucoadhesive delivery vehicles, in particular.

Mucoadhesive dosage forms have received attention as a novel drug delivery system to improve the bioavailability of drugs by prolonging the residence time and controlling drug release characteristics. These dosage forms, as yet, remain experimental. Thus far, the dosage forms which have been assembled and studied are tablets which contain a mucoadhesive polymer such as hydroxypropylmethylcellulose or Carbopol. Mucoadhesive formulations which have been tested include poly-hydroxyethyl-methacrylate microspheres coated with synthetic mucoadhesive polymers (C.-M. Lehr, *et al., J. Control. Rel.* **13**, 69 (1991)) and isohexylcyanoacrylate nanocapsules coated with poloxamers and poloxamine (C. Pimienta, *et al., Int. J. Pharm.* **80**, 1 (1992)).

Mucoadhesive liposomes have also been reported (H. Takeuchi, *et al., Chem. Phar. Bull.* **42**, 1954 (1994)). Liposomes, which were not themselves inherently mucoadhesive, were first formed and then subsequently coated with large polymers having mucoadhesive properties. The polymers used for liposome coating were chitosan, polyvinyl alcohol having a long alkyl chain and poly(acrylic acid) derivatized with cholesterol. These polymers had a weight range of between 137,000 to 250,000 daltons.

Importantly, these polymers were not incorporated into the lipid bilayer nor were small molecules such as glycosylceramides utilized.

There are currently no controlled transmucosal release devices that permit the maintenance of relatively constant plasma concentrations of drugs over extended periods of time, although a number of such devices have been developed for transdermal drug delivery. The transdermal devices include dramamine patches for treating motion sickness, nitroglycerin patches for angina, estradiol patches for hormone replacement therapy and nicotine patches for assistance in smoking cessation (Shaw *et al.*, PHYSIOLOGY, BIOCHEMISTRY AND MOLECULAR BIOLOGY OF THE SKIN, L.A. Goldsmith (Ed.) Oxford University Press, Oxford, 1991, pp. 1447-1501). The inherently greater permeability of the buccal mucosa (Lesch *et al., J. Dent. Res.* **68**, 1345 (1989)), its rich vascular supply (Squier, *et al., Arch. Oral Biol.* **30**, 313 (1985); Squier, *et al. Adv. Drug Delivery Rev.* **12**, 13 (1993)) and the ease of access of this surface suggests that the buccal mucosa is an ideal model mucosal surface with which to probe the transmucosal delivery of a wide range of pharmaceuticals to different mucosal surfaces throughout the body.

Preliminary studies (Ortale *et al., J. Dental Res.* **68**, 1345 (1993)) have indicated that liposomes prepared from lipids extracted from superficial layers of buccal epithelium adhere to and coat the buccal mucosal surface and are not readily rinsed away. However, it is unlikely that such extracted lipid liposomes can be prepared on a large enough scale to render them broadly useful as drug delivery vehicles. Nevertheless, liposomes incorporating such lipids can be used as supplemental therapy to replace barier lipids which have been damaged or depleted through aging or insult (e.g., chemotherapy, radiotherapy).

To achieve drug delivery through the mucosa, in general, and the buccal mucosa in particular, a means for maintaining a drug reservoir at the mucosa surface need to be developed. However, it is equally clear that the transdermal drug delivery patches currently in use cannot be readily adapted for use in the oral cavity. Therefore, there remains in the art a particular need for the development of improved compositions for use in oral drug, delivery. The development of a composition capable of delivering a drug to the systemic circulation following simple application to the mucosa surface would represent a significant advance. Quite surprisingly, the present invention provides such compositions and methods for using them.

SUMMARY OF THE INVENTION

The present invention generally provides a method for targeting drug delivery vehicles to mucosal surfaces by utilizing glycosylceramides as a targeting agent. Specifically, the present invention provides a broad range of mucoadhesive liposomal formulations. The liposomal formulations themselves are useful barrier repair lipid formulations. They can be used, for example, to improve moisturization and overall barrier protection. Additonally, the liposomes of the present invention can be loaded with encapsulated drug moieties and the encapsulated drugs thereby delivered to the mucosal surface onto which the liposomes adhere. The invention provides for drug delivery at the site of liposome adhesion for treatment of localized disorders and also for systemic delivery of the drug moieties via a transmucosal route. The use of very broad range of therapeutic agents is encompassed within the scope of the present invention. Drug moieties appropriate for use with the liposomes of the present invention include small molecules (*i.e.*, antibiotics, NSAIDS and the like), peptides (*i.e.*, insulin), oligonucleotides (*i.e.*, antisense, plasmids) and antigens and proteins (*i.e.*, vaccines).

Surprisingly, very small proportions of glycosylceramides can be used to produce mucoadhesive liposomes. This result is particularly significant to the large scale production of adherent liposomes for use in drug delivery, as glycosylceramides are the most expensive component of the liposomes.

Thus, in one aspect, the present invention provides a synthetic liposome comprising an amount of glycosylceramide which is effective to allow the formation of a vesicle that adheres to mucosa. In this aspect, the amount of glycosylceramide is high enough to allow the liposome to adhere to the mucosa and low enough to allow the liposome to form an intact vesicle.

In another aspect, the invention provides a method of encapsulating a bioactive agent in synthetic liposomes that adhere to mucosa comprising admixing the bioactive agent with an effective amount of glycosylceramide and an effective amount of a lipid such that the liposomes are formed.

In a further aspect, the invention provides a method for delivering a drug, which comprises the transmucosal delivery of a bioactive agent encapsulated in a liposome which adheres to the mucosa.

In yet another aspect, the invention provides a kit for preparing synthetic liposomes which adhere to mucosa. The kit comprises containers having an effective amount of a glycosylceramide and effective amount of a phospholipid.

In a still further aspect, the invention provides liposomes comprising a barrier lipid derived from a target mucosal surface.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings and micrographs form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings or micrographs in combination with the detailed description of specific embodiments presented herein.

FIG. 1. Glycosylceramide-containing liposomes form vesicles. Typical transmission electron micrograph of liposomes prepared from a phosphatidylcholine-glycosylceramide-cholesterol mixture (60-20-20).

FIG. 2. Electron micrographs of glycosylceramide-containing liposomes adhering to the buccal mucosa.

2A. Untreated buccal mucosa. Note the fine grooves of the cell surface.

2B. Buccal mucosa treated with liposomes prepared from phosphatidylcholine-glycosylceramides-cholesterol (79-1-20). The grooves are effectively coated by the treatment.

2C. Buccal mucosa treated with liposomes prepared from phosphatidylcholine-glycosylceramides-cholesterol (79-1-20) after three rinses with PBS. The grooves remain effectively coated.

2D. Buccal mucosa treated with liposomes containing no glycosylceramides (phosphatidylcholine-cholesterol, 80-20) after three rinses with PBS. The cell surface grooves are now evident.

FIG. 3. Electron micrograph of superficial buccal epithelium infected with *Candida albicans*.

3A. Control treated with phosphate buffered saline. Groove and valley surfaces of buccal mucosa are visible.

3B. Treated with liposomal ketoconazole. There are occasional individual *C. albicans*.

3C. Treated with ketoconazole solution. There are frequent colonies of *C. albicans*.

DETAILED DESCRIPTION OF THE INVENTION AND DESCRIPTION OF THE PREFERRED EMBODIMENTS

In addition to avoiding the risks associated with parenteral drug delivery and the metabolic barriers associated with oral delivery, slow release and systemic absorption of drugs from the present mucoadhesive liposomes offers the potential for maintaining a relatively constant systemic concentration of drug over an extended time period. Mucoadhesive liposomes are also uniquely suitable for maintaining effective drug concentrations within a mucosal surface at or near the site of liposome adherence.

Thus, in one aspect, the present invention provides a synthetic liposome comprising an amount of glycosylceramide which is effective to allow the formation of a vesicle that adheres to mucosa. In this aspect, the amount of glycosylceramide is high enough to allow the liposome to adhere to the mucosa and low enough to allow the liposome to form an intact vesicle.

The synthetic liposomes are designed to adhere to one or more mucosal surfaces including, for example, oral, gastric, intestinal, anal, vaginal, pulmonary, nasal, ocular, urethral, tracheal and/or esophageal mucosa. In a presently preferred embodiment, the liposomes adhere to oral, anal, pulmonary, nasal and/or ocular mucosa. In a still further preferred embodiment, the liposomes adhere to oral and/or anal mucosa. In yet another embodiment, the liposomes adhere to oral mucosa, particularly buccal mucosa.

The term "adhere" as used herein refers to an increased contact lifetime between a glycosylceramide-containing liposome of the invention relative to the contact lifetime of a liposome with an identical composition absent the glycosyl ceramide. "Contact lifetime," as used herein, defines the temporal duration of the contact between the liposoome and a mucosal surface. Any of a number of art-known *in vitro* and *in vivo* methods can be used to study the adherence of the liposomes of the invention. For example, hamster cheek pouches can be used as a model to study liposomal adherence to a mucosal surface (S.J. Sveinsson, *et al., Pharm. Res.* **9**, 1359 (1992)). Additionally, to evaluate the mucoadhesive function of the liposomes in vitro, a particle counting method using a Coulter counter can be used to measure the number of solution-phase liposomes remaining following incubation of a mucosal surface with the mucoadhesive liposomes (T.H. Yamamoto, *et al., Chem. Pharm. Bull.* **42**, 1954 (1994)). One of skill in the art will appreciate that modifications of these methods can be utilized to study the adhesive properties of the liposomes in vivo.

The incorporation into the synthetic liposomes of any glycosylceramide is within the scope of the present invention. In a preferred embodiment, the glycosylceramide is glucosylceramide, galactosylceramide or mixtures thereof.

Similarly, the incorporation of any useful lipid into the inventive liposomes is within the scope of the present invention.

As used herein, the term "lipid" refers to any fatty acid derivative, sterol or phospholipid which is capable of forming a bilayer such that a hydrophobic portion of the lipid material orients toward the bilayer while a hydrophilic portion orients toward the aqueous phase. Hydrophilic characteristics derive from the presence of phosphato, carboxy, sulfato, amino, sulfhydryl, nitro, and other like groups. Hydrophobicity is conferred by the inclusion of groups that include, but are not limited to, long chain saturated and unsaturated aliphatic hydrocarbon groups and such groups substituted by one or more aromatic, cycloaliphatic or heterocyclic group(s). Preferred lipids are phospholipids, including, for example, phosphoglycerides and sphingolipids, representative examples of which include phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyloleoyl phosphatidylcholine, lysophosphatidylcholine, lysophosphatidyl-ethanolamine, dipalmitoylphosphatidylcholine, dioleoylphosphatidylcholine, distearoyl-phosphatidylcholine or dilinoleoylphosphatidylcholine can be used. Other compounds

lacking in phosphorus, such as sphingolipid and glycosphingolipid families are also within the group designated as lipid. Additionally, the amphipathic lipids described above may be mixed with other lipids including, for example, triglycerides and sterols.

In a presently preferred embodiment, the synthetic liposomes of the invention incorporate one or more phospholipids. Examples of phospholipids which are presently preferred include, for example, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, sphingomyelin, lysophosphatidylcholine, lysophophatidylethanolamine, lysophosphatidylserine, lysophosphatidylinositol and lysophosphatidic acid or mixtures thereof. In a still further preferred embodiment, the invention provides synthetic liposomes containing phosphatidylcholine.

The liposomes of the invention can be formed utilizing glycosylceramides and lipids over a wide range of concentrations and relative proportions. The assembly of such differently formulated liposomes is well within the abilities of one of skill in the art.

This invention therefore provides liposomes prepared from lipid mixtures that resemble the properties that result from naturally occurring barrier lipids of buccal epithelial tissues to a sufficient degree to be of use in drug delivery across this tissue, but which liposomes can be simply and cost-effectively prepared using only a few different lipids each of which are readily available commercially. Table 1 lists preferred compositions that can be combined as indicated to comprise phospholipids, cerebrosides and sterols which are useful for the preparation of liposomal vesicles of the present invention. These lists are exemplary and not to be construed as comprehensive. The resultant liposomes are contemplated for use in oral drug delivery, particularly for large hydrophilic molecules such as peptides and small proteins.

TABLE 1. Liposome Components

Phospholipids	Glycosylceramides	Sterols
phosphatidylcholine	glucosylceramide	cholesterol
phosphatidylethanolamine	galactosylceramide	cholestanol
phosphatidylserine	any combination of the above	lanosterol
phosphatidylinositol		ergosterol
phosphatidic acid		stigmasterol
sphingomyelin		sitosterol
lysophosphatidylcholine		any combination of the above
lysophosphatidylethanolamine		
lysophosphatidylserine		
lysophosphatidylinositol		
lysophosphatidic acid		
any combination of the above		

In a presently preferred embodiment, the synthetic liposomes comprise at least about 1% glycosylceramide per total weight of lipid and an amount of phospholipid effective to allow formation of a vesicle which adheres to mucosa. In another embodiment, the synthetic liposomes comprise from about 1% to about 70% glycosylceramide per total weight of lipid. In a further embodiment, the synthetic liposomes comprise from about 1% to about 40% glycosylceramide per total weight of lipid. In yet another preferred embodiment, the synthetic liposomes comprise from about 1% to about 20% glycosylceramide per total weight of lipid. In a still further preferred embodiment, the synthetic liposomes comprise from about 1% to about 10% glycosylceramide per total weight of lipid.

The liposomes of the present invention can also be formulated to contain a sterol. Presently preferred sterols include, for example, cholesterol, cholestanol, lanosterol, ergosterol, stigmasterol and sitosterol. In a preferred embodiment, the sterol is cholesterol. The sterol is present in any useful concentration, however, in a preferred embodiment, it is present in an amount of at least 1% sterol per total weight of lipid. In another preferred embodiment, the sterol is present in an amount from about 1% to about 50% sterol per total weight of lipid. In a further preferred embodiment, the sterol is present in an amount from about 1% to about 30% sterol per total weight of lipid, more preferably 10% sterol per total weight of lipid.

The concentration ranges and relative proportions of the individual components of the inventive liposomes can be varied extensively and independently of each other. The only limitation is that the formulation must produce an intact liposome which exhibits mucoadhesive properties. It is within the scope of the present invention to "tune" the mucoadhesion of a particular liposome formulation by varying the identity and proportions of the individual constituents. Also within the scope of the invention is "tuning" the release rate of the agents which are encapsulated within the liposomes by a similar variation in formulation.

Liposomes of certain component proportions are presently preferred including from about 1% to about 20% sterol per total weight of lipid and from about 0.1% to about 70% glycosylceramide per total weight of lipid. In another preferred embodiment, the constituents are present in an amount from about 1% to about 10% sterol per total weight of lipid and from about 1% to about 70% glycosylceramide per total weight of lipid. In a further preferred embodiment, the constituents are combined in amounts of from about 1% to about 20% sterol per total weight of lipid and from about 1% to about 40% glycosylceramide per total weight of lipid. in yet another preferred embodiment, the liposomes comprise from about 1% to about 10% sterol per total weight of lipid and from about 1% to about 40% glycosylceramide per total weight of lipid.

In a presently preferred embodiment, the liposomes of the invention do not contain a pharmaceutically active moiety and are used therapeutically to replenish depleted or damaged barrier lipids. The function of barrier repair lipids is discussed in U.S. Patent No. 5,643,899 issued to Elias on July 1, 1997, which is incorporated herein by refeerence.

In another aspect, the invention provides a method of encapsulating a bioactive agent in synthetic liposomes that adhere to mucosa, the method comprising admixing the bioactive agent with an effective amount of sterol, an effective amount of glycosylceramide and an effective amount of a lipid such that the liposomes are formed.

The liposomes can encapsulate virtually any drug without limitation as to the drugs structure or activity. The only limitation on this encapsulation is that the drug and liposome constituents must be chemically compatible and the drug must be of an appropriate size to allow its encapsulation.

The liposomal compositions of the present invention provide a safe and effective amount of an active drug. As used herein, "safe and effective amount" means an amount of a drug which is high enough to significantly positively modify the condition to be treated, but low enough to avoid serious side effects within the scope of sound medical judgment. A safe and effective amount of a drug will vary with the specific drug, the ability of the drug to penetrate the mucosa, the amount of the liposomal composition applied to the mucosa, the particular condition being treated, the age and physical condition of the patient being treated, the severity of the condition, the nature of concurrent therapy and like factors.

The bioactive agents present in the liposomal formulations of the invention preferably comprise from about 0.01% to about 20% by weight of the compositions, more preferably from about 0.1% to about 5%. Mixtures of bioactive agents can also be encapsulated within the liposomes. It is contemplated herein that the various bioactive agents enumerated below can provide more than one benefit and can alternatively be classified under more than one category.

Useful drug activities in the compositions of the present invention include non-steroidal antiinflammatory drugs (NSAIDS). The NSAIDS can, for example, be selected from the following categories: propionic acid derivatives, acetic acid derivatives, fenamic acid derivatives, biphenylcarboxylic acid derivatives and oxicams. All of these NSAIDS are fully described in U.S. Patent No. 4,985,459 to Sunshine *et al.* issued Jan. 15, 1991, which is incorporated herein by reference. Most preferred are the propionic NSAIDS including, but not limited to aspirin, acetaminophen, naproxen, benoxaprofen, flurbiprofen, fenoprofen, fenbufen, ketoprofen, indoprofen, pirprofen, carprofen, oxaprozin, pranoprofen, miroprofen, tioxaprofen, suprofen, alminoprofen, tiaprofenic

acid, fluprofen and bucloxic acid. Also useful are the steroidal antiinflammatory drugs including hydrocortisone and the like.

Useful drug activities in the compositions of the present invention include antihistaminic drugs. Antihistaminic drugs preferred for inclusion in the present compositions include, for example, pharmaceutically acceptable salts of chlorpheniramine, triprolidine, diphenylhydramine, doxylamine, pyrilamine phenindamine, promethazine, cyproheptadine, azatadine, clemastine, carbinoxamine, tripelemamine, terfenadine, dexchlorpheniramine and mixtures thereof.

Useful drug activities in the compositions of the present invention include antitussive drugs. Antitussive drugs preferred for inclusion in the present compositions include, for example, pharmaceutically acceptable salts of dextromethorphan, codeine, carmiphen and carbetapentane.

Useful drug activities for inclusion in the compositions of the present invention include antipruritic drugs. Antipruritic drugs preferred for inclusion in the present compositions include, for example, pharmaceutically-acceptable salts of methidilizine and trimeprizine.

Useful drug activities for inclusion in the compositions of the present invention include anticholinergic drugs. Anticholinergic drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts of scopolamine, atropine, homatropine, levodopa, dicyclomine, hyoscyamine, procyclidine, trihexyphenidyl and ethopropazine.

Useful drug activities in the compositions of the present invention include anti-emetic and antinauseant drugs. Preferred drugs include, for example, pharmaceutically-acceptable salts of cyclizine, meclizine, chlorpromazine, buclizine, metoclopraminde, prochlorperazine and trimethobenzamide.

Useful drug activities for inclusion in the compositions of the present invention include anorexic drugs. Anorexic drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts of benzphetamine, phentermine, chlorphentermine, fenfluramine, diethylpropion and phendimetrizine.

Useful drug activities for inclusion in the compositions of the present invention include central stimulant drugs. Central stimulant drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically

acceptable salts of amphetamine, methamphetamine, dextroamphetamine and methylphenidate.

Useful drug activities for inclusion in the compositions of the present invention include antiarrhythmic drugs. Antiarrythmic drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts of propanolol, procainamide, disopyraminde, quinidine, encainide, flecanaide, mexiletine and tocainamide. Other preferred antiarrythmic drugs include pharmaceutically acceptable salts of the quinidine derivatives disclosed in U.S. Pat. No. 4,716,171 issued to Jareau and Koenig on Dec. 29, 1987, which is incorporated herein by reference.

Useful drug activities for inclusion in the compositions of the present invention include β-adrenergic blocker drugs. β-adrenergic blocker drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts metoprolol, acebutolol, betaxolol, labetalol and timolol. β-adrenergic blocker drugs more preferred for inclusion in the compositions of the present invention include metoprolol tartrate, acebutolol hydrochloride, betaxolol hydrochloride, labetalol hydrochloride and timolol maleate.

Useful drug activities for inclusion in the compositions of the present invention include cardiotonic drugs. Cardiotonic drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts milrinone, amrinone and dobutamine. Other cardiotonic drugs preferred for inclusion in the present compositions include pharmaceutically-acceptable salts of 14-aminosteroid derivatives, some of which are disclosed in U.S. Patent Nos. 4,325,879, 4,552,868 and 4,584,289 issued to Jareau and Koenig on April 20, 1982, Nov. 12, 1985 and Apr. 22, 1986, respectively, each of which are incorporated herein by reference.

Useful drug activities for inclusion in the compositions of the present invention include antihypertensive drugs. Antihypertensive drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts of enalapril, clonidine, hydralazine, minoxidil, guanadrel, guanethidine, guanfacine, mecamylamine, methyldopate, pargyline, phenoxybenzamine and prazosin.

Useful drug activities for inclusion in the compositions of the present invention include diuretic drugs. Diuretic drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts of

amiloride and hydrochlorothiazide. Diuretic drugs more preferred for incorporation into the present compositions include amiloride hydrochloride.

Useful drug activities for inclusion in the compositions of the present invention include vasodilator drugs. Vasodilator drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts of diltazem, amiodarone, isosuprine, nylidrin, tolazoline, verapamil, nitroglycerin and other nitric oxide donors.

Useful drug activities for inclusion in the compositions of the present invention include vasoconstrictor drugs. Vasoconstrictor drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts of dihydroergotamine, ergotamine and methylsergide.

Useful drug activities for inclusion in the compositions of the present invention include antiulcer drugs. Antiulcer drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts of ranitidine and cimetidine.

Useful drug activities for inclusion in the compositions of the present invention include anesthetic drugs. Anesthetic drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts of lidocaine, bupivacaine, chlorprocaine, dibucaine, etidocaine, mepivacaine, tetracaine, dyclonine, hexylcaine, procaine, cocaine, ketamine, pramoxine and phenol.

Useful drug activities for inclusion in the compositions of the present invention include antidepressant drugs. Antidepressant drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts of imipramine, desipramine, amitryptiline, nortryptiline, protryptiline, doxepin, maprotiline, phenelzine, tranylcypromine, trazodone and trimipramine.

Useful drug activities for inclusion in the compositions of the present invention include tranquilizer and sedative drugs. Tranquilizer and sedative drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts of chlordiazepoxide, benacytyzine, benzquinamide, flurazapam, hydroxyzine, loxapine and promazine.

Useful drug activities for inclusion in the compositions of the present invention include antipsychotic drugs. Antipsychotic drugs which are preferred for

incorporation into the present composition include, for example, pharmaceutically acceptable salts of chlorprothixene, fluphenazine, haloperidol, molindone, thioridazine and trifluoperazine.

Useful drug activities for inclusion in the compositions of the present invention include antimicrobial drugs (antibacterial, antifungal, antiprotozoal and antiviral drugs). Antimicrobial drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts of β-lactam drugs, quinolone drugs, ciprofloxacin, norfloxacin, tetracylcine, erythromycin, amikacin, triclosan, doxycycline, capreomycin, chlorhexidine, chlortetracycline, oxytetracycline, clindamycin, ethambutol, hexamidine isothionate, metronidazole, pentamidine, gentamycin, kanamycin, lineomycin, methacycline, methenamine, minocycline, neomycin, netilmycin, paromomycin, streptomycin, tobramycin, miconazole and amanfadine. Antimicrobial drugs preferred for inclusion in the present compositions include tetracycline hydrochloride, erythromycin estolate, erythromycin stearate (salt), amakacin sulfate, doxycycline hydrochloride, oxatetracycline hydrochloride, clindamycin hydrochloride, ethambutol hydrochloride, metronidazole hydrochloride, pentamidine hydrochloride, gentamicin sulfate, kanamycin sulfate, lineomycin hydrochloride, methacycline hydrochloride, methenamine hippurate, methenamine mandelate, minocycline hydrochloride, neomycin sulfate, netilmicin sulfate, paromomycin sulfate, streptomycin sulfate, tobramycin sulfate, miconazole hydrochloride, amanfadine sulfate, triclosan, octopirox, parachlorometaxylenol, nystatin, tolnaftate and clotrimazole.

Useful drug activities for inclusion in the compositions of the present invention include antineoplastic drugs. Antineoplastic drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts of anti-androgens (*e.g.*, leuprolide or flutamide), cytocidal agents (*e.g.*, adriamycin, doxorubicin, taxol, cyclophosphamide, busulfan, cisplatin, α-2-interferon) anti-estrogens (*e.g.*, tamoxifen), antimetabolites (*e.g.*, fluorouracil, methotrexate, mercaptopurine, thioguanine), *etc.* The functional group component can also comprise hormones (*e.g.*, medroxyprogesterone, estradiol, leuprolide, megestrol, octreotide or somatostatin).

Useful drug activities for inclusion in the compositions of the present invention include muscle relaxant drugs. Muscle relaxant drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically

acceptable salts of cinnamedrine, cyclobenzaprine, flavoxate, orphenadrine, papaverine, mebeverine, idaverine, ritodrine, dephenoxylate, dantrolene and azumolen.

Useful drug activities for inclusion in the compositions of the present invention include antispasmodic drugs. Antispasmodic drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts of the antispasmodic compounds disclosed in U.S. Pat. No. 3,856,825 issued to Wright et al. on Dec. 24, 1974, which is incorporated herein by reference.

Useful drug activities for inclusion in the compositions of the present invention include antidiarrheal drugs. Antidiarrheal drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts of loperamide.

Useful drug activities for inclusion in the compositions of the present invention include bone-active drugs. Bone-active drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts of diphosphonate drug compounds and phosphonoalkylphosphinate drug compounds including the prodrug esters thereof. Such compounds are disclosed, for example, in U.S. Pat. Nos. 3,683,080, 4,304,734, 4,687,768, 4,711,880 and 4,719,203, each of which are incorporated herein by reference.

Useful drug activities for inclusion in the compositions of the present invention include endocrine modulating drugs. Endocrine modulating drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts of contraceptives (*e.g.*, ethinodiol, ethinyl estradiol, norethindrone, mestranol, desogestrel, medroxyprogesterone), modulators of diabetes (*e.g.*, glyburide or chlorpropamide), anabolics, such as testolactone or stanozolol, androgens (*e.g.*, methyltestosterone, testosterone or fluoxymesterone), antidiuretics (*e.g.*, desmopressin) and calcitonins can also be incorporated into the compositions. Estrogens (*e.g.*, diethylstilbesterol), glucocorticoids (*e.g.*, triamcinolone, betamethasone) and progenstogens, such as norethindrone, ethynodiol, norethindrone, levonorgestrel, ethinylestradiol can be incorporate into the compositions. In yet other embodiments, thyroid agents (*e.g.*, liothyronine or levothyroxine) or anti-thyroid agents (*e.g.*, methimazole) can be used. In further embodiments, hyperprolactinemic disorders can be treated using compositions containing, for example, cabergoline. The use of hormone suppressors (*e.g.*, danazol or goserelin), oxytocics (*e.g.*, methylergonovine or oxytocin)

and prostaglandins, such as mioprostol, alprostadil or dinoprostone, can also be employed in the compositions.

Useful drug activities for inclusion in the compositions of the present invention include immunomodulating drugs. Immunomodulating drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts of antihistamines, such as benadryl, loratadine, brompheniramine, periactin, promethazine, terfenadine, fexofenadine, azelastine and/or clemastine. In still another embodiment, the compositions can contain mast cell stabilizers, such as lodoxamide and/or cromolyn.

Other components which modulate the immune system include, but are not limited to, steroids (*e.g.*, triamcinolone, beclomethazone, cortisone, dexamethasone, prednisolone, methylprednisolone, beclomethasone, or clobetasol), immunostimulants (*e.g.*, imiquimod), histamine H_2 antagonists (*e.g.*, famotidine, cimetidine, ranitidine), immunosuppressants (*e.g.*, azathioprine, cyclosporin, tacrolimus, ascomycin, rapamycin, 5-aminosalicylic acid, N-acetylcysteine, methotrexate, retinoids, vitamin D3 and its analogs). Groups with anti-inflammatory activity, such as antioxidants, sulindac, etodolac, ketoprofen and ketorolac, can also be incorporated into the compositions.

Useful drug activities for inclusion in the compositions of the present invention include urinary tract drugs. Urinary tract drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts of uricosuric agents (*e.g.*, sulfinpyrazone, indanyl carbenicillin, nitrofurantoin, nolidixic acid, neomycin, bacitracin and polymyxin B), antispasmodics (*e.g.*, oxybutynin and flavoxate) and calcium oxalate stone preventatives. The compositions can also include a drug for the treatment of prostatic hypertrophy (*e.g.*, terazosin or finasteride). In another embodiment, the composition is used for treating cystitis and contains, for example, pentosan.

Useful drug activities for inclusion in the compositions of the present invention include ophthalmic drugs. Ophthalmic drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts of β-blockers (*e.g.*, brominide or betaxolol), antiinflammatories (*e.g.*, clopatadine), useful in the treatment of glaucoma (*e.g.*, latanoprost) and carbonic anhydrase inhibitors, such as dichlorphenamide, methazolamide or dorxolamide.

Useful drug activities for inclusion in the compositions of the inveniton include peptide-based drugs (*e.g.*, Lutenizing hormone releasing hormone, vasopressin and insulin)

Useful drug activities for inclusion in the compositions of the present invention incldrugs ude dental drugs. Dental drugs which are preferred for incorporation into the present composition include, for example, fluoride sources and pharmaceutically acceptable salts amlexanox for the treatment of aphthous ulcers.

In still further embodiments, the liposomes of the present invention contain an agent which enhances the permeation of encapsulated drug molecules or liposome components into the mucosa. Useful permeation enhancers include, for example, hydrophilic enhancers such as glycerol and poly(ethyleneglycol) of various molecular weights, DMSO and the like. Alos within the scope of the present invention is the use of hydrophobic permeation enhancers such as fatty acids including oleic acid, lauric acid, myristic acid, stearic acid; di- and tri-glycerides such as sorbitan monooleate, sorbitan trioleate, and glycerol monolineolate; di- and tri-esters of sorbitols such as sorbitan monooleate, sorbitan trioleate and sorbitan monooleate, isopropyl myristate and sucrose monococoate.

The permeation enhancers are present in the formulations of the invention in a permeation enhancing amount, that is, in an amount effective to enhance the permeation of an active agent or liposome component. This will depend on several factors, such as the particular active agent or liposome component, but is generally in an amount of from about 0.001 wt % to about 50 wt %, preferably from about 0.1 wt % to about 10 wt %.

In a further aspect, the invention provides a method for delivering a drug, which comprises the transmucosal delivery of a bioactive agent encapsulated in a liposome which adheres to the mucosa.

In yet another aspect, the invention provides a kit for preparing synthetic liposomes which adhere to mucosa comprising containers having an effective amount of a glycosylceramide and effective amount of a phospholipid.

In a still further aspect, the invention provides mucoadhesive liposomes comprising a barrier lipid derived from a target mucosal surface. The compositions of these liposomes are substantially similar to those liposomes discussed above. The barrier lipids can be isolated by extraction of a sample of mucosa or by other means which are

known to those of skill in the art. Alternatively, the barrier lipid can be synthetically produced. The stucture of the barrier lipids can be identical to the endogenous barrier lipid or they can have structural differences. The structural differences can be at sites remote from the site necessary for barrier lipid recogntion of the mucosa or proximate to these sites. The sturctural differences can be included either to "tune" the binding or drug release properties of the liposomes or, in the case of synthetic liposomes, to simplify or reduce the cost of the synthetic route.

In a presently preferred embodiment, the liposomes with a barrier lipid contain a pharmaceutically active moiety and serve as mucoadhesive drug delivery vehicles. In another preferred embodiment, the liposomes do not contain a pharmaceutically active moiety and are used therapeutically to replenish depleted or damaged barrier lipids

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLES

To achieve mucoadhesive liposomes in a practical manner, it was initially necessary to demonstrate that liposomes with properties similar to those made from a representative mucosal surface could be prepared from simple mixtures of commercially available lipids. The buccal mucosa was chosen as an appropriate experimental model. Although the remaining examples focus on the use of the buccal mucosa as an

experimental model, it is not intended that the invention be thereby limited to use with the buccal mucosa. It will be plain to those of skill in the art that techniques and compositions similar to those disclosed below will be equally appropriate for use with other mucosal surfaces.

The major lipid classes in superficial buccal epithelium are phospholipids (52% of total lipid mass), glycosylceramides (14%) and cholesterol (17%). Liposomes with properties resembling those prepared from buccal epithelial lipids were prepared from a mixture of the major lipid components, without the cost and difficulty of duplicating the exact total lipid composition. Example 1 details the preparation of liposomes formed from buccal barrier lipids.

Having demonstrated that liposomes with a composition analogous to that of the buccal mucosa could be prepared, the ability of the liposomes to adhere to the buccal mucosa was investigated. Example 2 details experiments, using transmission electron microscopy, to investigate the mucoadhesion of the liposomes

Example 3 illustrates the formation of liposomes from mixtures of commercially available lipids. The effect on vesicle formation of a reciprocal variation of the proportions of phospholipid and glycosylceramide was investigated. As the proportions of these components were varied, the cholesterol concentration was held constant at 0%, 10% or 20%.

Examples 4 and 5 illustrate the characterization of the properties of the liposomes. Electron microscopy of negative stained specimens revealed small unilamellar vesicles with an diameters of 1-1.5 μm. When applied to buccal mucosa, these liposomes filled in grooves and coated the epithelial surface as revealed by scanning electron microscopy, and this coating was not removed by at least three rinses with PBS.

In Example 6, the effects of varying the proportions of the sterol were investigated. When the proportion of sterol was fixed at 20%, it was found that mixtures containing between 1% and 39% glycosylceramides formed intact liposomes. Mixtures containing 40% or more glycosylceramides failed to form liposomes. All of the preparations containing 1% to 39% glycosylceramides adhered to mucosa and were not removed by repeated washing in PBS.

In still further studies, the role of sterols in liposome formation and mucoadhesion was examined. Cholesterol was used as an exemplary sterol in these investigations. The proportion of cholesterol was fixed at either 0% or 10% and the

proportion of glycosylceramide and phospholipid was varied. Surprisingly, as the proportion of cholesterol was decreased, the proportion of glycosylceramide that could be incorporated, while still forming intact vesicles, increased at greater than a linear rate. For example, mixtures containing 10% cholesterol and from 1% up to and including 60% glycosylceramides formed mucoadhesive liposomes, and mixtures containing 0% cholesterol and from 1% up to and including 70% glycosylceramides also formed liposomes that were mucoadhesive.

This latter result, that mucoadhesive liposomes could form without any sterol present, was in direct contrast to what would be expected based upon the composition of buccal lipids and earlier results which indicated that cholesterol was both necessary for liposome formation and was also a limiting factor regarding the incorporation of other types of lipids into the liposome.

Thus, it was found that, under appropriate conditions, mucoadhesive liposomes require only two of the three major buccal lipid classes to form and do not necessarily need to closely mimic the natural buccal lipid composition. Thus it was concluded that liposomes made from either 0% sterol, 1% to 70% glycosylceramides and phospholipid; 10% sterol, 1% to 60% glycosylceramides and phospholipid; or 20% sterol, 1% to 39% glycosylceramides and phospholipid will be useful for the sustained release of materials at a mucosal surface, *i.e.*, in drug delivery vehicles.

In Example 5, the mucoadhesion of the liposomes of Example IV is illustrated.

Example 6 illustrates the effect on mucoadhesion of varying the glycosylceramide concentration.

Example 7 demonstrates the finding that liposomes without glycosylceramides do not show significant mucoadhesion. For the nonkeratinized regions of the oral mucosa, glycosylceramides are a key component for the preparation of mucoadhesive liposomes. This may reflect a specific recognition of the glycoside head group of the glycolipid by a lectin-like component of the epithelial cell. A glycosylceramide content of about 15% by weight approximates to that found in the actual buccal barrier (Law *et al., Arch. Oral Biol.* **40**, 1085 (1995)). An exemplary formulation which closely mimics the lipid composition in the superficial layers of the nonkeratinized oral mucosae comprises 20% cholesterol, 15% glycosylceramides and 65% phosphatidylcholine.

In the presence, or even the absence, of any sterol, mucoadhesive liposomes will form with as little as 1% glycosylceramides. If, however, to much glycosylceramide is incorporated into the mixture, intact vesicles will not form. This is illustrated in Example 8.

Examples 9 and 10 illustrate the use of other liposomal components and suggest the storage of the liposomes of the present invention by freeze-drying, respectively.

Example 11 demonstrates that the liposomal formulations of the invention can deliver drugs, in their active form, to the mucosa to which they adhere. The liposomes were used to deliver ketoconazole, a broad spectrum antifungal agent, to a sample of porcine buccal mucosa.

EXAMPLE 1

PREPARATION AND COMPOSITION OF BUCCAL BARRIER LIPIDS

The purpose of this initial study was to determine whether liposomes could be prepared from buccal barrier lipids. Porcine buccal barrier was isolated by digestion with trypsin. Lipids were extracted from the isolated barrier and analyzed by thin-layer chromatography.

The lipid content of the buccal barrier preparation is shown in Table 2.

TABLE 2. Lipid Content of the Buccal Barrier

Lipid Components	Weight Percent	
	Epidermal stratum corneum	Buccal barrier
Phospholipids	0.0	50.2
Cholesterol sulfate	2.4	4.8
Glycosylceramides	0.8	11.7
Ceramides	52.5	0.6
Cholesterol	26.7	16.6
Fatty acids	10.0	8.8
Triglycerides	0.0	3.8
Cholesterol esters	7.6	3.5

Epidermal stratum comeum lipids consist primarily of ceramides, cholesterol and fatty acids, whereas the buccal barrier lipids consist mainly of phospholipids, glycosylceramides and cholesterol.

Table 2 also compares the buccal barrier lipids with those of epidermal stratum corneum. This comparison is provided as just one example of the different lipid compositions that can be found in different barriers of the body. Despite the significant differences in the lipid compositions in Table 2, the inventors found that small unilamellar vesicles also could be prepared from the stratum corneum lipids. It is important to note that the results from the stratum corneum lipid study established that the stratum corneum lipids can form stable liposomal vesicles even in the absence of glycosylceramides (Wertz *et al, J. Invest. Dermatol.* 87, 582 (1986)). A similar approach can be applied to prepare liposomal formulations designed to target other mucosal surfaces.

EXAMPLE 2

BUCCAL BARRIER LIPID LIPOSOMES

The barrier lipid was suspended in phosphate buffered saline (PBS, pH 7.4) and sonicated for 55 minutes at 45°C. Transmission electron microscopy of negative stained specimens revealed small unilamellar vesicles with an average diameter of 0.2 μm. When applied to buccal mucosa, these liposomes filled in the grooves and

fissures and coated the epithelial surface as revealed by scanning electron microscopy, and this coating was not removed by at least three rinses with PBS.

EXAMPLE 3

PREPARATION OF SYNTHETIC LIPID MIXTURES

Liposomes and lipid mixtures were prepared from commercially available (lipids purchased from Sigma Chemical Company, St. Louis MO). These included soy bean phosphatidylcholine (phosphatidylcholine), glycosylceramides (galactosylceramide from bovine brain or glycosylceramide from Gaucher's spleen and cholesterol.

Individual lipids were dissolved in chloroform:methanol, 2:1, at a concentration of 2 mg/ml. Lipid solutions were transferred to 16 x 120 mm glass screw cap culture tubes so that each tube contained 10 mg total lipids with the desired proportions of each. cholesterol was fixed at 0, 10% and 20% by weight, respectively, and the proportions of phosphatidylcholine and glycosylceramide were varied reciprocally so that the following glycosylceramide proportions were included: 0, 1.0, 10, 20, 30, 35, 37.5, 40, 60, 70 and 80%. Lipids were dried under a gentle stream of nitrogen at 45°C to produce a thin film of lipid on the walls of the tube. After rehydration with 10 ml of phosphate buffered saline (PBS), the mixture was sonicated at 50°C for 55 min, annealed at 50°C for 15 minutes and cooled to room temperature.

EXAMPLE 4

EXEMPLARY SYNTHETIC LIPOSOMAL VESICLE FORMATION

4.1 Electron Microscopy

Liposomal preparations were examined by transmission electron microscopy after negative stamina with phosphotungstic acid. A portion of liposomal suspension was diluted 1:4 with 3% glutaraldehyde and 3% formaldehyde in 0.1 M cacodylate buffer. One drop of the fixed liposome suspension was transferred to each of three grids followed by application of a drop of 2% phosphotungstic acid at pH 7. After 3 minutes, excess solution was removed from the grids with a paper wick, and the grids were allowed to dry for an additional 3 hours prior to examination with a Zeiss EM 10 transmission electron microscope.

4.2 Results

FIG. 1 shows a transmission electron micrograph of negatively stained liposome formulated to resemble buccal barrier lipids in composition in a general sense. The glycosylceramide content in natural buccal barrier lipids is about 14%. The exemplary mixture of liposomes, used in the study represented in FIG. 1, was prepared from a phosphatidylcholine-glycosylceramide-cholesterol mixture of 60-20-20.

FIG. 1 shows that, as with liposomes prepared from authentic buccal barrier lipids, the present commercially available lipid mixture of 60-20-20 phosphatidylcholine-glycosylceramide-cholesterol resulted in the formation of small unilamellar vesicles with diameter of approximately 1-1.5 μm.

EXAMPLE 5

EXEMPLARY SYNTHETIC LIPOSOMAL BUCCAL ADHERENCE

5.1 Application to Mucosa

Liposome suspensions were applied to the surface of small (ca. 5 x 5 mm) pieces of fresh porcine buccal mucosal tissue with a Pasteur pipet. Some of the treated mucosa was then rinsed by immersion in 25 ml of PBS up to three times.

5.2 Electron Microscopy of Buccal Mucosa

Buccal mucosa with or without liposome treatment was fixed in 3% glutaraldehyde and 3% formaldehyde in 0.1 M cacodylate buffer at pH 7.3 for 1-2 hours. Excess fixative was removed by rinsing twice for ten minutes each with 0.1 M cacodylate buffer. The specimens were then dehydrated through a series of graded ethanol solutions (10, 50, 70, 90, 2 x 95 and 2 x 100%) for 10 minutes each. The samples were air dried prior to mounting on stubs and sputtercoating with gold/palladium prior to examination with an Amray 1820 scanning electron microscope.

The exemplary liposome preparation of commercially available lipids in the mixture of 60-20-20 (phosphatidylcholine-glycosylceramide-cholesterol), which formed small unilamellar vesicles (see Example IV), was also found to adhere to the buccal mucosa.

EXAMPLE 6

RANGE OF GLYCOSYLCERAMIDE LIPOSOMAL ADHERENCE

6.1 Mixtures Containing 20% Sterol

Using the techniques of mucosal application and electron microscopy, as described above, other formulations of glycosylceramide-containing lipid mixtures were tested for buccal adherence. It was found that in the presence of 20% cholesterol each of the mixtures containing between 10% and 20% glycosylceramide formed liposomes and adhered to buccal mucosa.

It was also found that mixtures containing between 21% and 39% glycosylceramide formed liposomes and adhered to the mucosal surface. The ability of liposomes containing between 21% and 39% glycosylceramide to adhere to the mucosa represents a wide range of effectiveness in adherent properties, considering that natural superficial buccal epithelia contain only 14% glycosylceramide.

Surprisingly, it was found that each of the mixtures containing between 1% and 10% glycosylceramide also formed liposomes and also adhered to mucosa. In fact, even glycosylceramide-containing liposomes with phosphatidylcholine-glycosylceramide-cholesterol ratios of 79-1-20 readily attached to the buccal mucosal surface and were not removed by repeated rinsing. This is demonstrated by comparing the fine grooves of the cell surface seen in FIG. 2A with FIG. 2B and FIG. 2C, in which the grooves are effectively coated by the treatment.

6.2 Mixtures Containing less than 20% Sterol

Further investigation of mixtures that contained less than 20% cholesterol provided several surprising results. Mixtures were prepared as previously; however, the sterol content was kept constant at either 10% cholesterol or 0% cholesterol and the glycosylceramide and phosphatidylcholine content was varied.

When mixtures containing greater than 39% glycosylceramide were examined, it was found that mucoadherent liposomes were formed under the new conditions. In fact, mixtures which contained only 10% cholesterol formed mucoadhesive liposomal vesicles with from 1% up to 60% glycosylceramide and mixtures that contained 0% cholesterol formed mucoadhesive liposomal vesicles with from 1% up to 70% of the liposome comprised of glycosylceramide.

Given the known buccal epithelium content of 14% glycosylceramide, the inventors' findings that liposomes with as low as 1% glycosylceramide or as high as 70% had the ability to form vesicles and to adhere to the relevant surface were unexpected. In contrast to this finding, one would have presumed from that the effective glycosylceramide concentration would mirror the natural concentration considerably more closely (Ortale *et al. J. Dent. Res.* **74**, 59 (1995)) .

Even more surprising is the result that no sterol needs to be present in order for a mucoadherent liposome to be formed. This result is unexpected since it would have been predicted that cholesterol was necessary for membrane fluidity and structure.

EXAMPLE 7

GLYCOSYLCERAMIDES ARE ESSENTIAL FOR ADHERENCE

Using the same techniques of mucosal application and electron microscopy described above, it was found that glycosylceramide are a necessary component of the liposome if mucoadherence is to be achieved substantively.

FIG. 2D shows that the buccal mucosa treated with liposomes containing no glycosylceramides (phosphatidylcholine-cholesterol, 80-20) has evident cell surface grooves after three rinses with PBS. This result indicates that the liposomes without glycosylceramide did not adhere to the mucosal surface with sufficient strength to resist removal upon the addition of the buffer. Such liposomes would not therefore be good candidates for oral drug delivery.

The critical nature of glycosylceramides in adherence could not have been predicted from the inventors' earlier studies on buccal barrier lipids. For example, given the range of compounds identified in Table 2 and the data of Example II, there was no indication that the absence of one particular lipid compound would completely destroy the ability of a liposome to adhere to the buccal mucosa. This is particularly surprising as the addition of a mere 1% of the absent compound (glycosylceramide) is sufficient to completely restore the strong adherent property.

EXAMPLE 8

Excess Gycosylceramides Prevent Liposome Formation

Although the presence of glycosylceramides in liposomes is essential for buccal adherence, and although lipid mixtures containing concentrations of between 1% and 39% glycosylceramide are capable of forming liposomal vesicles at any concentration of cholesterol examined, formulations which contained 40% or more glycosylceramide only formed liposomes under certain circumstances. These results were seen using electron microscopy.

For example, when the sterol content was reduced from 20% to 10%, the glycosylceramide content in a mixture could be as much as 60% and still form liposomes. But higher concentrations of glycosylceramide failed to form liposomes. Yet if the sterol content was again reduced, in the exemplary case from 10% to 0% cholesterol, then the glycosylceramide content could be as much as 70% and liposomes could be formed. Under no circumstances did mixtures contained 71% or more glycosylceramide form liposomes.

EXAMPLE 9

Further Liposomal Components

Comparison of the attached buccal barrier liposomes with those prepared from other lipids suggests that the attachment is not based on electrostatic interaction but may represent binding at specific sites on the tissue surface.

In addition to glycosylceramide, the liposomal formulation must include a charged or bipolar bilayer-forming lipid, for example, phosphatidylcholine, and may include a sterol, for example, cholesterol. Other phospholipids and/or sterols may also be present in the liposomal formulation. Other exemplary phospholipids and sterols that can be used in the present invention are listed in Table 1. These liposomal components may be used alone or in any combination with each other.

EXAMPLE 10

Preservation and Storage of Liposomes

It has been reported that liposomes in general do not have a long shelf life, and so should be prepared shortly before use. This has seriously limited the use of liposomes in a wide range of potential therapeutic applications. However, there is a

growing literature indicating that liposomes can be preserved by freeze drying and reconstituted with water when needed (Sachse *et al., Invest. Radiol.* **28**, 838 (1993); Friede *et al., Ana. Biochem.* **211**, 117 (1993); Cannon *et al., Pharm. Res.* **10**, 715 (1993)).

EXAMPLE 11

DELIVERY OF LIPOSOMALLY ENCAPSULATED KETOCONAZOLE

11.1 Materials and Methods

Ketoconazole (200 mg) was dissolved in 20 mL of phosphate buffered saline (ketoconazole solution). Half of this solution was added to a tube containing a lipid mixture consisting of phosphatidylcholine (35 mg), glucosylceramide (5 mg) and cholesterol (10 mg). This mixture was sonicated for 15 minutes at 45 °C and then allowed to cool to room temperature. A 0.35 μm-thick sheet of superficial buccal epithelium was cut from porcine buccal mucosa. The cut surface of this sheet was glued to a plastic backing using cyanoacrylate and one cm diameter disks were cut using a biopsy punch.

Treatment of disks and incubation were performed in a multiwell plate. Four disks were included in each of three treatment groups. One group was treated with 1 mL of phosphate buffered saline (PBS) for five minutes. The second group was treated with 1 mL of the ketoconazole solution for five minutes. The third set was treated with 1 mL of liposomal ketoconazole for five minutes. After five minutes, the excess liquid was removed from each of the samples and each disk was rinsed with PBS (3 x 1 mL). Each disk was then overlayed with 1 mL of YEPD culture medium containing 10^5 *Candida albicans* yeast. After incubation for 24 hours at 37 °C, the disks were rinsed with PBS and fixed with 2.5% glutaraldehyde in cacodylate buffer at 4 °C for 24 hours. The disks were then dehydrated through a graded series of acetone solutions, sputter coated with gold and examine with an Amray scanning electron microscope.

11.2 Results

The control group of tissue disks showed the typical groove and valley surface of buccal epithelium. The liposomal ketoconazole group showed filling of the grooves and an occasional individual *Candida*. The ketoconazole solution group showed frequent colonies of *Candida albicans*.

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the composition, methods and in the steps or in the sequence of steps of the methods described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents that are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

WHAT IS CLAIMED IS:

1. A synthetic liposome comprising a lipid and glycosylceramide in na amount such that said liposome adheres to mucosa.

2. The synthetic liposome according to claim 1, wherein said mucosa is a member selected from the group consisting of oral, gastric, intestinal, anal, vaginal, pulmonary, nasal, ocular, urethral, tracheal, esophageal mucosa and mixtures thereof.

3. The synthetic liposome according to claim 2, wherein said mucosa is a member selected from the group consisting of oral, anal, pulmonary, nasal, ocular and mixtures thereof.

4. The synthetic liposome according to claim 3, wherein said mucosa is a member selected from the group consisting of oral and anal mucosa and mixtures thereof.

5. The synthetic liposome according to claim 4, wherein said mucosa is oral mucosa.

6. The synthetic liposome according to claim 5, wherein said mucosa is buccal mucosa.

7. The synthetic liposome according to claim 1, wherein said glycosylceramide is a member selected from the group consisting of glucosylceramide, galactosylceramide and mixtures thereof.

8. The synthetic liposome according to claim 2, wherein said lipid is a phospholipid.

9. The synthetic liposome according to claim **8**, wherein said phospholipid is a member selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, sphingomyelin, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylserine, lysophosphatidylinositol, lysophosphatidic acid and mixtures thereof.

10. The synthetic liposome according to claim **9**, wherein said lipid is phosphatidylcholine.

11. The synthetic liposome according to claim **8** comprising at least about 1% glycosylceramide per total weight of lipid and an amount of phospholipid effective to allow formation of a vesicle which adheres to mucosa.

12. The synthetic liposome according to claim **11** comprising from about 0.1% to about 70% glycosylceramide per total weight of lipid.

13. The synthetic liposome of claim **12** comprising from about 1% to about 40% glycosylceramide per total weight of lipid.

14. The synthetic liposome of claim **13** comprising from about 1% to about 20% glycosylceramide per total weight of lipid.

15. The synthetic liposome of claim **14** comprising from about 1% to about 10% glycosylceramide per total weight of lipid.

16. The synthetic liposome according to claim **1** further comprising a sterol.

17. The synthetic liposome according to claim **16**, wherein said sterol is a member selected from the group consisting of cholesterol, cholestanol, lanosterol, ergosterol, stigmasterol and sitosterol.

18. The synthetic liposome according to claim 17, wherein said sterol is cholesterol.

19. The synthetic liposome according to claim 16 comprising at least about 1% sterol per total weight of lipid.

20. The synthetic liposome according to claim 19 comprising from about 1% to about 50% sterol per total weight of lipid.

21. The synthetic liposome according to claim 19 comprising from about 1% to about 30% sterol per total weight of lipid.

22. The synthetic liposome according to claim 21 comprising from about 1% to about 10% sterol per total weight of lipid.

23. The synthetic liposome according to claim 21 comprising from about 0.1% to about 70% glycosylceramide per total weight of lipid.

24. The synthetic liposome according to claim 22 comprising from about 1% to about 10% sterol per total weight of lipid and from about 1% to about 70% glycosylceramide per total weight of lipid.

25. The synthetic liposome according to claim 23 comprising from about 1% to about 20% sterol per total weight of lipid and from about 1% to about 40% glycosylceramide per total weight of lipid.

26. The synthetic liposome according to claim 23 comprising from about 1% to about 10% sterol per total weight of lipid and from about 1% to about 40% glycosylceramide per total weight of lipid.

27. A method for treating damaged epithelium, said method comprising contacting said epithelium with a liposome according to claim 1.

28. The synthetic liposome according to claim **1** further comprising a bioactive agent encapsulated within the liposome.

29. The synthetic liposome according to claim **28**, wherein said bioactive agent is a member selected from the group consisting of non-steroidal antiinflammatory agents, antihistaminic agents, antitussive agents, antipruritic agents, anticholinergic agents, anti-emetic agents, antinausea agents, anorexic agents, central stimulants, antiarrhythmic agents, B-adrenergic blocking agents, cardiotonic agents, antihypertensive agents, diuretic agents, vasodilating agents, vasoconstricting agents, antiulcer agents, anesthetic agents, antidepressant agents, tranquilizers, sedatives, antipsychotic agents, antimicrobial agents, antineoplastic agents, muscle relaxing agents, antispasmodic agents, antidiarrheal agents, bone-active agents, endocrine modulating agents, immunomodulating agents, urinary tract agents, ophthalmic agents, dental agents and mixtures thereof.

30. The synthetic liposome according to claim **29**, wherein said bioactive agent is fluoride.

31. The synthetic liposome according to claim **29**, wherein said bioactive agent is an immunomodulatory agent.

32. The synthetic liposome according to claim **29**, wherein said bioactive agent is an antimicrobial agent.

33. The synthetic liposome according to claim **29**, wherein said bioactive agent is an anesthetic agent.

34. The synthetic liposome according to claim **29**, wherein said bioactive agent is an antineoplastic agent.

35. The synthetic liposome according to claim **28**, wherein said bioactive agent is a member selected from the group consisting of peptides and DNA sequences.

36. A pharmaceutical formulation comprising the synthetic liposome according to claim **1** and a pharmaceutically acceptable excipient.

37. A mouthwash comprising a population of synthetic liposomes according to claim **1**, wherein said liposomes contain an encapsulated bioactive agent.

38. A method of making a synthetic liposome according to claim **1** comprising admixing an effective amount of a lipid with an effective amount of a glycosylceramide to form an intact liposome that adheres to mucosa.

39. The method according to claim **38**, wherein said amount of glycosylceramide is from about 1% to about 70% per total weight of lipid.

40. The method according to claim **39**, wherein said amount of glycosylceramide is from about 1% to about 30% per total weight of lipid.

41. The method according to claim **40**, wherein said amount of glycosylceramide is from about 1% to about 10% per total weight of lipid.

42. A method of encapsulating a bioactive agent in a synthetic liposome that adheres to mucosa, said method comprising admixing said bioactive agent with an effective amount of a glycosylceramide and an effective amount of a lipid, thereby encapsulating said bioactive agent.

43. A method for transmucosal delivery of a bioactive agent to a subject, said method comprising contacting a mucosal surface of said subject with a liposome according to claim **1**.

44. A method for transmucosal delivery of a bioactive agent to a subject, said method comprising contacting a mucosal surface of said subject with a mucoadhesive liposome according to claim **28**.

45. The method according to claim 44, wherein said drug is used to treat a disease state in said subject.

46. A kit for preparing synthetic liposomes which adhere to mucosa comprising containers having an effective amount of a glycosylceramide and an effective amount of a lipid.

47. The kit according to claim 46, further comprising a bioactive agent.

48. A liposome prepared from a barrier lipid, said lipid being derived from a mucosal surface.

49. A liposome according to claim 48 further comprising a bioactive agent.

50. A method for treating damaged epithelium, said method comprising contacting said epithelium with a therapeutically effective amount of a liposome according to claim 48.

51. A method for targeting a liposome to a specific epithelial surface in a subject, said method comprising administering a liposome according to claim 48 to said subject.

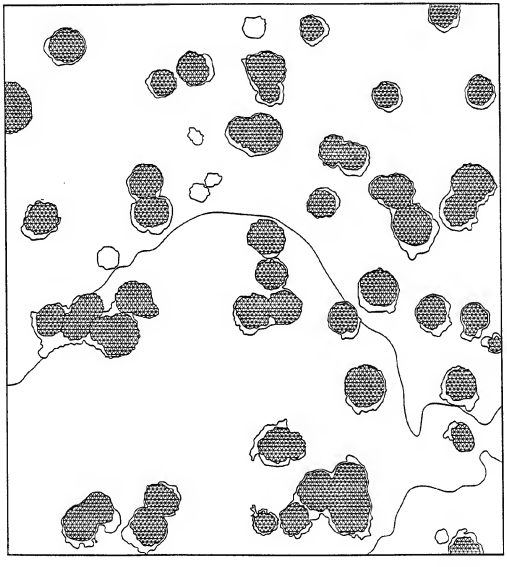

FIG. 1

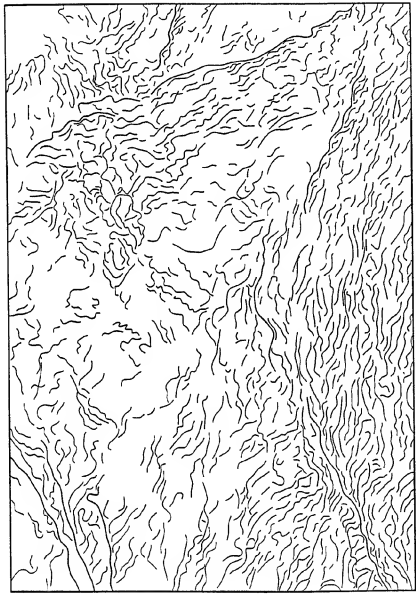

Fig. 2a

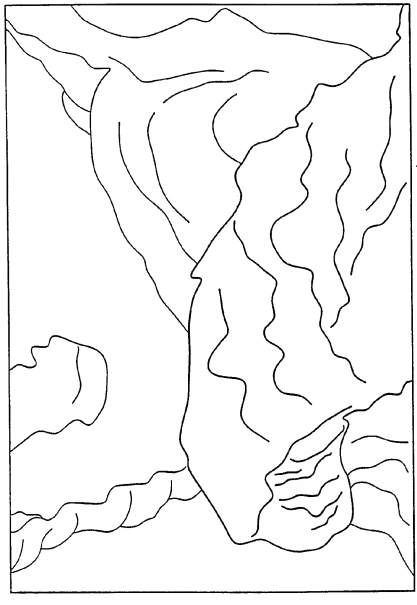

Fig. 2b

Fig. 2c

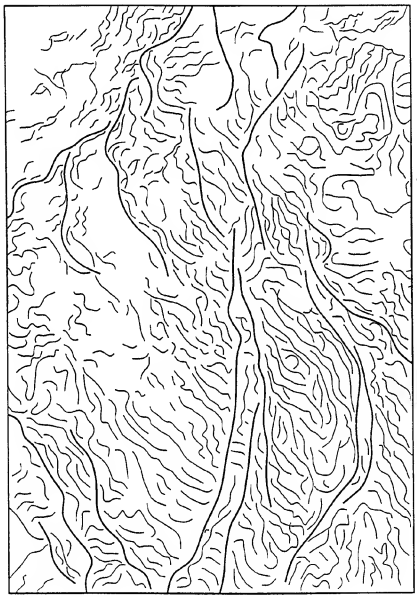

Fig. 2d

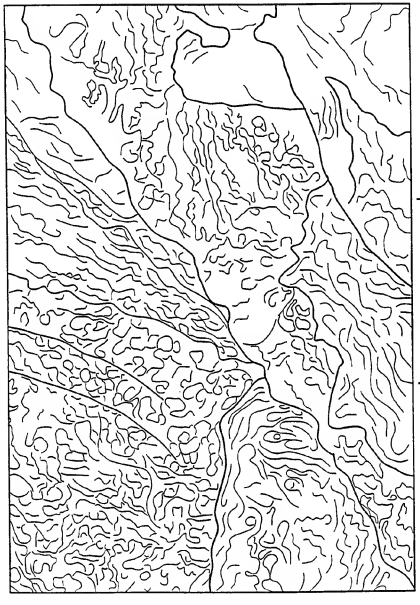

Fig. 3a

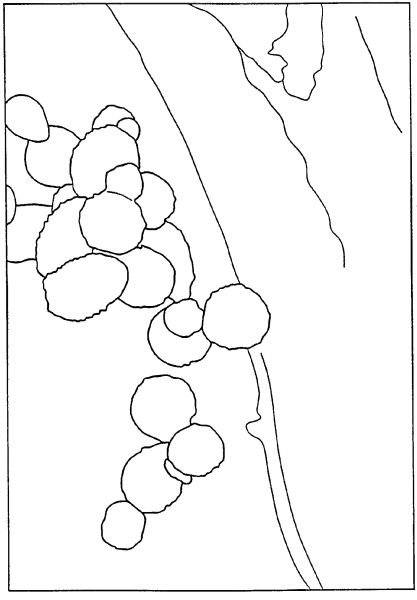

Fig. 3b

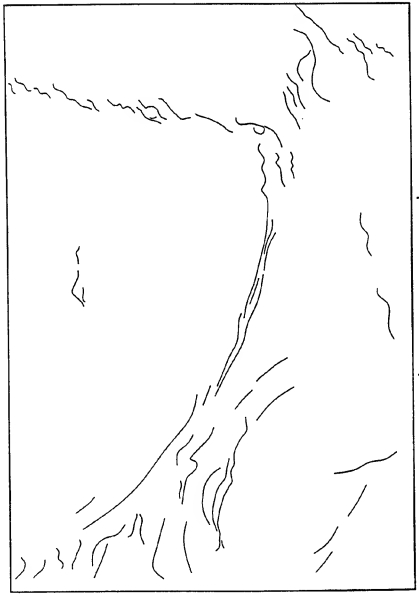

Fig. 3c

INTERNATIONAL SEARCH REPORT

Intern. .tal Application No
PCT/US 98/06457

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/127 A61K9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4 416 872 A (ALVING CARL R ET AL) 22 November 1983 see the whole document ---	1-18,28, 29,32, 36, 38-42, 46,47
A	PATENT ABSTRACTS OF JAPAN vol. 012, no. 373 (C-534), 6 October 1988 & JP 63 126820 A (SHISEIDO CO LTD), 30 May 1988, see abstract ---	
A	EP 0 351 808 A (SEARLE & CO) 24 January 1990 ---	
A	US 5 242 800 A (JIMENEZ VICTOR E ET AL) 7 September 1993 -----	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 July 1998	16.07.98
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Fischer, W

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/06457

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 27,43,45,50,51
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Although claims 27,43,45,50,51 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Claims Nos.: 27,43,45,50,51

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna...al Application No
PCT/US 98/06457

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4416872 A	22-11-1983	NONE	
EP 0351808 A	24-01-1990	US 5073543 A JP 2124830 A	17-12-1991 14-05-1990
US 5242800 A	07-09-1993	NONE	